Journal of Medical Virology 60:300–312 (2000)

The HIV-2 Genotype and the HIV-1 Syncytium-Inducing Phenotype Are Associated With a Lower Virus Replication in Dendritic Cells

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During sexual transmission, HIV infects the mucosal dendritic cells and is transferred to CD4 T cells. Whether HIV variants of a particular genetic (sub)type or phenotype selectively infect dendritic cells (DC) or are preferentially transferred to T cells remains highly controversial. To avoid the cumbersome use of primary dendritic cells, in vitro dendritic cell models were generated from precursors, either hematopoietic progenitor cells (HPC) or monocytes (MO). Productive infection in the dendritic cells and transfer of the virus to T cells was assessed for a range of HIV variants. HPC-derived dendritic cells (HPC-DC) were more susceptible to HIV-1 than to HIV-2 isolates. The HIV-1 group O strains were more productive in HPC-DC than group M, but amongst the latter, no subtype-related difference was observed. Both non-syncytium-inducing (NSI) and SI HIV isolates and lab strains could productively infect HPC-DC, albeit with a different efficiency. Adding blocking antibodies confirmed that both CCR-5 and CXCR-4 co-receptors were functional. Biological HIV-1 clones of the NSI/R5 phenotype infected more readily HPC-DC than SI/X4 clones. MO-derived dendritic cells were, however, more exclusive in their preference for NSI/R5 clones. Some HIV variants, that did not grow readily in HPC-DC alone, could be rescued by adding resting or pre-activated T cells. The present data show that HIV-2 isolates and SI clones replicate less in model-DC, but no preference for a particular HIV-1 subtype was evident. Co-culture with T cells could "correct" a limited growth in dendritic cells. Clearly, both intrinsic dendritic cell susceptibility and enhancement by T cells are explained only partly by HIV genotype and phenotype. The in vitro dendritic cell models seem useful tools to further unravel interactions between HIV, DC, and T cells. ***J. Med. Virol. 60:300–312, 2000.***

KEY WORDS: dendritic cells; HIV-2; HIV-1 group O; HIV-1 group M; non syncytium-inducing; syncytium-inducing

INTRODUCTION

Dendritic cells (DC) in the genital and rectal mucosa are amongst the first targets of the human immune deficiency virus (HIV) during sexual transmission [Zambruno et al., 1995; Spira et al., 1996; Masurier et al., 1998]. The interaction between infected DC and T cells induces activation of the latter, resulting in HIV-specific immune responses, [Knight et al., 1991; Manca et al., 1994], but it also favors HIV transfer to CD4 T cells [Cameron et al., 1992; Pope et al., 1994; Ayehunie et al., 1995; Pope et al., 1995]. The balance between early infection of T cells, promoting HIV dissemination,

Abbreviations: BCS, bovine calf serum; CD, cluster of differentiation; DC, dendritic cell; FCS, fetal calf serum; GM-CSF, granulocyte-monocyte colony-stimulating factor; HPC, hematopoietic progenitor cell; HPC-DC, hematopoietic progenitor cell-derived dendritic cell; IL, interleukin; LC, Langerhans cell; MO, monocyte; MO-DC, monocyte-derived dendritic cell; MO-MA, monocyte-derived macrophage; NSI, non syncytium-inducing; PBMC, peripheral blood mononuclear cells; PHA, phytohemagglutinin; R5, chemokine receptor CCR5-using; SCF, stem cell factor; SI, syncytium-inducing; X4, chemokine receptor CXCR4-using.

Grant sponsor: FWO Vlaanderen; Grant number: 3.0226.96.

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Accepted 30 August 1999

and anti-HIV responses, attempting to control the infection, is probably a key determinant in the course of the disease [Macatonia et al., 1990; Kahn and Walker 1998].

The epidemiology of various HIV types and subtypes differs geographically and evolves over time. In West Africa HIV-2 was predominant originally, but over 90% of the new infections are now caused by HIV-1 [Kanki et al., 1997; Tarantola and Schwartländer , 1997]. One of the possible, but unexplored, explanations is a preferential susceptibility of DC for HIV-1 over HIV-2. The homosexual epidemic in Europe and North America involves largely HIV-1 subtype B, whereas the principal subtype in the early heterosexual spread in Thailand was E. A higher intrinsic susceptibility of Langerhans cells for subtype E over B was suggested as a biological explanation [Soto-Ramirez et al., 1996], but this hypothesis is highly controversial [Dittmar et al., 1997; Pope et al., 1997].

The various HIV isolates display inter-related phenotypic characteristics, including their co-receptor usage (CXCR-4 vs. CCR-5), syncytium-inducing capacity (SI vs. NSI) and cellular tropism (T cell- vs. MO-tropic) [Björndal et al., 1997; Dittmar et al., 1997; Bazan et al., 1998; Hoffman and Doms, 1998]. These characteristics change during the course of the disease: from almost exclusively R5/NSI/MO-tropic soon after infection to a higher prevalence of X4/SI/T-tropic clones in advanced disease [Schuitemaker, 1994; Conner et al., 1997; Glushakova et al., 1998; Spijkerman et al., 1998; Tersmette et al., 1988]. The early phenotype could be explained by selection of R5/NSI clones from the infecting heterogeneous HIV "swarm" or quasispecies in the first target cells, including the DC and interacting T cells, or by more efficient elimination of X4/SI clones by the intact immune system [Reece et al., 1998].

Whether DC are infected preferentially by particular HIV variants remains controversial. Some authors claim that DC are highly susceptible to all kinds of HIV strains and clones and that both CCR-5 and CXCR-4 are functionally present, whereas others have shown a clear-cut preference of DC for the R5/NSI strains [Langhoff et al., 1991; Chehimi et al., 1993; Cameron et al., 1994; Ludewig et al., 1995; Patterson et al., 1995; Cameron et al., 1996; Blauvelt et al., 1997; Zaitseva et al., 1997; Carr et al., 1998; Reece et al., 1998; Rubbert et al., 1998; Spijkerman et al., 1998; Zoetweij et al., 1998]. Several factors could account for this discrepancy, including the heterogeneity of DC in vivo, the problems of obtaining sufficient numbers of primary DC for extensive testing, the almost inevitable contamination with T cells and monocytes, the limited survival of primary DC in vitro and the different conditions of infection used in these studies.

Many of the limitations of primary dendritic cells can be overcome by generating dendritic cells in vitro from precursors. Two major pathways have been discovered. Dendritic cells can be derived directly from CD34(+) hematopoietic progenitor cells (HPC) [Caux et al., 1992; Santiago-Schwartz et al., 1992; Herbst et al., 1996; Rosenzwaijg et al., 1996; Szabolcs et al., 1996; Lardon et al., 1997] or from peripheral MO [Pickl et al., 1996; Romani et al., 1994; Sallusto and Lanzavecchia et al., 1994; Romani et al., 1996]. Millions of these "model DC" can be generated, without contaminating CD4 T cells and they show good viability over several weeks in vitro. In the present study, these in vitro models have been used to compare their susceptibility to a wide range of HIV variant, including primary HIV-1 group M, HIV-1 group O and HIV-2 strains as well as pairs of HIV-1 clones with opposite co-receptor usage and syncytium-inducing capacity. In addition, the effect on virus production of adding back CD4 T cells to infected DC cultures was investigated. The results indicate that HIV-2 is less dendrotropic than HIV-1 and that NSI clones tend to infect DC more readily than SI counterparts. Co-culture of virus-pulsed DC with resting or activated T cells, however, could at least overcome in part restrictions of viral growth in DC.

MATERIALS AND METHODS

Immuno-Phenotyping of Various Cell Populations

Phenotyping was carried out as described previously [Lardon et al., 1997]. The following monoclonal antibodies were purchased from Becton Dickinson (Erembodegem, Belgium): FITC- and PE-labeled isotypic controls, anti-CD3-FITC, anti-CD4-PE, anti-CD13-PE, anti-CD14-FITC, anti-CD14-PE and anti-CD80-PE. The anti-CD1a-FITC was obtained from Ortho (Beerse, Belgium), anti-CD1a-PE from Caltag (San Francisco, CA), anti-CD13-FITC from DAKO (Glostrup, Denmark), anti-CD40-FITC from Biosource (Zoersel, Belgium) and anti-CD86-PE from Pharmingen (San Diego, CA). Acquisition and analysis were carried out on a FACScan flow cytometer, manufactured by Becton Dickinson.

Generation of Dendritic Cells From Hematopoietic Progenitor Cells

Mononuclear cells were isolated from cord blood using Lymphocyte Separation Medium (ICN Biomedicals, Costa Mesa, CA). The cells were labeled indirectly with anti-CD34 and selected on a FACStar-plus cell sorter (Becton Dickinson, Erembodegem, Belgium). The purity of CD34(+) cells was routinely >95%. These CD34(+) HPC were cultured at 10^5/ml in 2 ml IMDM (Gibco BRL, Paisley, Scotland), with 10% foetal calf serum (Sera Lab, Sussex, UK), 1% bovine serum albumin (Sigma, Bornem, Belgium), penicillin 100 U/ml and Streptomycin 100 μg/ml (both from Gibco BRL, Paisley, Scotland). This culture medium was supplemented with cytokines according to Herbst et al., [1996] and our own modifications [Van Tendeloo et al., 1998]. Briefly, the HPC were first expanded for 8 days in 100 ng/ml interleukin-3 (IL-3, kindly provided by Dr. S.C. Clark, Genetic Institute, Cambridge, MA), 100 ng/ml IL-6 (Boehringer-Mannheim, Mannheim, Germany) and 50 ng/ml stem cell factor (SCF also from Boehringer-Mannheim). Over two weeks, these cells were

differentiated into DC during their incubation in 100 ng/ml granulocyte-monocyte stimulating factor (GM-CSF from Boehringer-Mannheim) and 1000 U/ml IL-4 (Genzyme, Cambridge, MA). In some experiments 2.5 ng/ml tumour necrosis factor-alpha (TNF-α from Boehringer-Mannheim) was added during the last week of culture. The DC generated with GM-CSF + IL-4 are referred to as "precursor HPC-DC," those pretreated with GM-CSF + IL-4 + TNF as "TNF-differentiated HPC-DC."

Generation of Dendritic Cells and Macrophages From Monocytes

The technique for the differentiation of DC from monocytes (MO) was based on that of Sallusto and Lanzavecchia [1994] and Romani et al., [1994]. Six hundred million mononuclear cells (PBMC), isolated from buffy coats, obtained from the Antwerp Red Cross Blood Transfusion Centre, were subjected to counterflow elutriation, using the J6-MC elutriator from Beckman (Beckman Instruments Inc., Palo Alto, CA), according to the protocol of Bruyninckx et al., [1990]. The fractions with > 80% MO, identified by flowcytometry as CD4(+) CD3(−), were pooled and subjected to sheep erythrocyte (E) rosetting [Coligan et al., 1992]. The E-rosette-negative population contained 90–95% MO, 3–5% B cells and NK cells. The T cell contamination was consistently <0.5%. Thirty to eighty million of these highly enriched MO were cultured for 5–7 days in complete medium in the presence of either 20 ng/ml GM-CSF (kindly provided by Schering Plough, Dardilly, France) alone or together with 20 ng/ml IL-4 (R & D Systems Europe, Abingdon, UK). Half of the medium, containing the same cytokines, was replaced twice during the culture period. MO treated with GM-CSF only differentiated into macrophages (MA), whereas the cells cultured with GM-CSF + IL-4 developed DC characteristics. These cell populations will be referred to as MO-MA and MO-DC respectively.

Activated and Resting T Cells

Activated T cells were obtained by stimulating PBMC in complete medium with 1 μg/ml phytohemagglutinin (PHA) from Murex (Abbott, Wiesbaden, Germany) for 3 days and incubating them further for 1–2 days in medium with 10 U/ml IL-2 (from Boehringer-Mannheim). This population is referred to as PHA T blasts. Resting CD4 T cells were purified from fresh PBMC by positive selection with Dynabeads (Dynal, Oslo, Norway). They contained >97% CD3(+) CD4(+) and <0.5% MO.

In-House HIV Antigen Capturing Assay

To determine the HIV-1 concentration in the culture supernatants, an in-house ELISA was used, the technical details of which have been described recently [Beirnaert et al., 1998]. The in-house test recognizes all HIV-1 group M and O isolates and clones with a lower threshold of 200 pg/ml, using the HIV p24 viral lysate concentrate from Dupont (Wilmington, DE) as a standard.

To quantify HIV-2 in the supernatants, this in-house test was modified, by using a broadly specific capture antibody and the conjugate from the Innotest (Innogenetics, Gent, Belgium), that recognises both HIV-1 and HIV-2. Using the same Dupont p24 viral lysate standard as for the HIV-1 ELISA, we showed that the HIV-2 assay has a lower threshold of 62.5 pg/ml.

As the purpose of our study was to distinguish viral isolates and clones in a qualitative way (significant growth in DC or not), a standard curve was not used systematically, but only a positive and a negative control were included in triplicate. The negative samples resulted in a mean optical density (O.D.) 0.061 (±0.017), similar to the wells without added sample. Based on this observation, the O.D. of the tested samples was corrected by subtracting 0.1 from the observed O.D. value and these corrected O.D.s are presented in the tables and figures.

HIV Strains and Clones

Two laboratory-adapted viruses (HTLV-III_B and Ba-L) were kindly provided by the NIH AIDS Research and Reference Reagent Program (Rockville, MD). The 23 primary isolates and 8 clones were from our own collection: 11 isolates are HIV-1 group M [Delwart et al., 1993; Janssens et al., 1994; Nkengasong et al., 1994; Heyndrickx et al., 1998), 4 are HIV-1 group O [Nkengasong et al., 1997] and 8 are HIV-2 [Nyambi et al., 1997]. Of the 4 pairs of clones, 2 are HIV-1 subtype B and 2 are subtype D [Zhong et al., 1995].

Viral stocks were grown in PHA T blasts and stored as supernatants in aliquots at −80°C. The infectious titre of these stocks was quantified as tissue culture infectious dose 50 (TCID50) in PHA T blasts. To this end, 4 duplicates of a three-fold dilution series in 100 μl RPMI were prepared in a 96-well flat bottom plate (Sterilin, Stone Staffs, UK). One hundred μl PHA T blasts at 7.5×10^5/ml in RPMI, containing 15% FCS and 10 U/ml of IL-2 (Boehringer-Mannheim), were added to each well. The trays were incubated for 2 hours at 37°C. The cells were then washed three times, resuspended in IL-2 containing medium and further incubated at 37°C. Release of HIV antigen into the supernatant of each well was measured after 7 days. The TCID50 was calculated according to the method of Reed and Muench [1938], as modified by Peden and Martin [1995].

Subtype was established by phylogenetic analysis of the *env* gene sequences, using TREECON software [Van de Peer and De Wachter, 1994]. Syncytium-inducing capacity was assessed by culturing virus isolates or clones in the MT-2 cell line [Nkengasong et al., 1995]. Co-receptor usage was determined in CD4- and co-receptor expressing U87 cells [Björndal et al., 1997] and in a second assay, using GHOST cells, transfected with CD4, one of the coreceptors and LTR-driven enhanced green fluorescent protein [Cecilia et al., 1998]. Five thousand MT-2, U87 and GHOST cells were in-

fected with the virus variants at a multiplicity of infection (MOI) of 10^{-2} and 10^{-3} in duplicate. After overnight incubation, the cells were washed three times. The cells were cultured for 14 days, when the release of HIV antigen into culture supernatant was determined. The supernatants from cell lines without added virus were negative in ELISA. (O.D. <0.1). The lab strains Ba-L and HTLV-III_B were used as positive and negative controls. Supernatants from Ba-L-infected CCR-5 cells had a high antigen concentration, whereas those from similarly exposed CXCR-4 or MT-2 cultures were negative. HTLV-III_B infection showed the reverse patterns.

Conditions of Infection of Dendritic Cells and Macrophages

Viral stocks was diluted in RPMI to give 10^3 or 10^4 TCID50/ml. They were filtered through a 0.2 μm filter (Sartorius AG, Göttingen, Germany) and incubated with 0.1 U of RNAse-free DNAse (from Boehringer) per 1 TCID50 for 30 minutes at room temperature. The MA or DC, at 10^7/ml in RPMI with 10% FCS, were dispensed into aliquots in 15 ml polypropylene tubes and mixed with a suitable volume of the diluted virus to obtain the desired multiplicity of infection (ranging from 10^{-2} to 10^{-4} MOI). In the first series of experiments (summarized in Fig. 1) "limiting" conditions of infection were used: the precursor HPC-DCs were incubated with the virus at 3×10^{-3} MOI for 30 minutes at 4°C. In all other experiments, more "permissive" conditions were used: the TNF-differentiated HPC-DC, the MO-MA and MO-DC were incubated with the virus (at 10^{-2}, 10^{-3} or 10^{-4} MOI) for 2 hours at 37°C. After infection, the cells were washed 5 times with 10 ml RPMI to remove any free virus and resuspended at 2×10^6/ml in 10% BCS-RPMI.

Culture Conditions to Induce Virus Production

Five hundred μl cultures were performed in a 48 well plate. Each condition was set up in duplicate and replicated once or twice in independent experiments, with cells from different donors. The cultures containing only DC (or MA) were set up with a final concentration of 10^6 cells per ml. The DC or MA were cultured in complete RPMI medium (with 10% BCS and antibiotics), that was further supplemented with GM-CSF at 20 ng/ml (= GM-CSF medium). Over the next 3 weeks, half of the supernatant was harvested for HIV antigen detection and replaced with the same medium twice weekly. In some experiments, co-cultures of DC and allogeneic T cells (either resting or PHA T blasts) were undertaken in parallel with the cultures of DC alone. T cells were added either at a late stage of incubation (experiments of Fig. 1) to rescue potentially latent virus. Alternatively, T cells were added immediately after infection (experiment of Fig. 4), to maximally promote virus transfer from DC to T cells, even before proviral DNA could be integrated in the DC.

In the experiments of Figure 1, four duplicate cultures of precursor HPC-DC (in GM-CSF medium) were set up immediately after infection. After removal of 250 μl of the supernatant on the 9th day of culture, only 2 of the 4 duplicates were replenished with GM-CSF medium. Allogeneic PHA T blasts (250 μl at 10^6/ml final concentration)were added to the 2 remaining duplicates. The DC-T cell co-cultures were maintained on medium containing 10 U/ml IL-2.

In the experiments, depicted in Figure 4, threefold dilutions of TNF-differentiated HPC-DC, ranging from 330×10^3 to 0.15×10^3 cells/ml, were (1) incubated with GM-CSF medium only (= DC alone) or (2) co-cultured with allogeneic PHA T blasts and maintained in IL-2 medium, or (3) co-cultured with allogeneic resting CD4 T cells in complete medium without cytokines. Both sets of T cells were added at 10^6/ml (final concentration). The latter co-cultures of resting CD4 T cells and DC were replenished after 3 days with medium lacking cytokines (to allow the DC to have an allo-stimulatory effect on the T cells) and subsequently these co-cultures were replenished with IL-2 containing medium (to maintain T cell viability).

Two kinds of negative controls were included: (1) uninfected DC and (2) co-cultures of PHA T blasts with the rinsing fluid of last wash after virus inoculation. DC infected with Ba-L (10^{-3} MOI) were the positive controls. All duplicate bi-weekly supernatants were frozen at −20°C and at the end of the culture period, the HIV antigen content was measured. If antigen production occurred, the concentration always rose with time. The results, represented in the tables and figures, are limited to the HIV-antigen concentration at the end of the 3 weeks culture period.

Co-Receptor Blocking Experiment

Ba-L at 3×10^{-4} MOI or HLTV-III_B at 3×10^{-3} MOI were inoculated on the TNF-differentiated HPC-DC, that had previously been dispensed into either medium alone or medium, containing monoclonal antibodies to CCR-5 (2D7) or CXCR-4 (12G5). Both mAbs were obtained from the NIH AIDS Research and Reference Reagent Program (Rockville, MD) and used at 10 μg/ml. After incubation for 2 hours at 37°C and extensive washing, the DC were cultured in GM-CSF medium, containing either no antibody or 10 μg/ml of the same anti-chemokine-receptor antibody as used during the infection. The antibodies were added again after 3 days, but from Day 5 on, GM-CSF-containing medium without antibodies was used for all cultures.

RESULTS

Characteristics of HIV Strains and Clones

The genotypes and phenotypes of the virus isolates and clones are summarized in Table I.

Eleven HIV-1 strains were NSI and exclusively used

TABLE I. Genotypic and Phenotypic Characteristics of the HIV Variants Studied

	Env subtype[a]	Syncytium inducing capacity[b]	Co-receptor usage[c]	
			U87	GHOST
HIV-1 group M lab strains				
Ba-L	B	NSI	R5	R5
$HTLV\text{-}III_B$	B	SI	X4	X4
HIV-1 group M isolates				
VI 1380	A	NSI	R5	R5
CI 22	B	NSI	R5	R5
VI 1923	B	NSI	R5	R5
VI 829	C	NSI	R5	R5
VI 1401	D	NSI	R5	R5
CI 13*	D	NSI	R5	R5
VI 824	D	NSI	R5	R5
VI 820	D	SI	R5 + X4	R5 + X4
VI 1888	E	NSI	R5	R5
CA 10*	E	SI	X4	X4
VI 1249	E	SI	R5	R5
HIV-1 group O isolates				
VI 70*	O	NSI	R5	R5
VI 686*	O	NSI	R5	R5
CA 9*	O	NSI	R5	R5
VI 878	O	SI	X4	X4
HIV-2 isolates				
VI 53	A	NSI	R5	R5
VI 905	A	NSI	No growth	No growth
VI 1415	A	NSI	R5	R5
VI 1835	ND	NSI	R5	R5
VI 884	A	SI	R5 + X4	R5
CI 197	ND	SI	X4	X4
CBL 20	ND	SI	X4	X4
CI 85	ND	SI	R5 + X4	X4
HIV-1 biological clones				
VI 886-1	B	NSI	R5	R5
VI 886-6	B	SI	R5 + X4	R5 + X4
VI 943-4	B	NSI	R5	R5
VI 943-1	B	SI	X4	X4
VI 761-1	D	NSI	R5	R5
VI 761-4	D	SI	X4	X4
VI 820-1	D	NSI	R5	R5
VI 820-4	D	SI	X4	X4

ND = not determined.
[a]*Env* subtypes were determined using TREECON software [Van de Peer and De Wachter, 1994]. The isolates marked with * were classified using full-length gp160 sequencing. In the others, partial sequencing was performed, as described in Janssens et al. [1994].
[b]The syncytium-inducing capacity was deduced from growth of the isolate/clone in MT-2 cells [Nkengasong et al., 1995].
[c]The co-receptor usage was determined in parallel in the CCR-5 and CXCR-4 expressing U87 [Björndal et al., 1997] and the GHOST cells [Cecilia et al., 1998]. One isolate (VI 905) failed to grow in all cell lines at the maximum concentration used (10^{-2} MOI).

CCR-5. Two of the 4 primary SI isolates used CXCR-4 exclusively, one used CCR-5 and one could use either co-receptor.

Four of 8 HIV-2 isolates were NSI: 3 of these used CCR-5, although the co-receptor use of VI 905 remains undetermined, because it failed to grow in either U87 or GHOST cells at 10^{-2} MOI (the maximal concentration used in these experiments). Of the four SI HIV-2 strains, two used CXCR-4 exclusively and the two others (VI 884 and CI 85) could use either co-receptor in U87 cells. The latter two produced a discrepant result in the GHOST cells, in that VI 884 used only CCR-5 and CI 85 only CXCR-4.

Out of the 4 pairs of biological HIV-1 clones, two were subtype B and two subtype D: all 4 NSI clones used the CCR-5 co-receptor, and 3 out of the 4 SI clones used CXCR-4 exclusively, whereas the 4th SI clone (VI 886-6) could use either co-receptor.

Characteristics of Macrophages and Dendritic Cells

Macrophages and DC were generated from purified MO in 5 experiments. MO-DC typically expressed CD1a in a high proportion, whereas CD14 was almost exclusively present on MO-MA (paired t-test $P < 10^{-3}$). CD4 and CD13 expression were similar in both cell types. Of the co-stimulatory molecules, CD40 and CD80 expression was similar in MA and DC, but CD86 was higher in the MO-DC (Table II). MO-DC thus represents a model of immature DC, resembling dermal dendritic cells [Grassi et al., 1998].

In 12 experiments, DC were generated from cord

TABLE II. Phenotype of In Vitro Generated Dendritic Cells and Macrophages

Cells of origin	Peripheral blood monocytes (MO)		Hematopoetic progenitors cells (HPC)	
Differentiating cytokines	GM-CSF only	GM-CSF + IL-4	GM-CSF + IL-4	GM-CSF + IL-4 + TNF-α
Differentiated cell type	MO-MA	MO-DC	Precursor HPC-DC	TNF-differentiated HPC-DC
CD1a	4 ± 1[a]	70 ± 9[a]	37 ± 15[b]	51 ± 24[b]
CD4	77 ± 16	79 ± 2	47 ± 13	38 ± 14
CD13	98 ± 3	98 ± 2	94 ± 4	96 ± 3
CD14	90 ± 10[a]	8 ± 4[a]	24 ± 6[b]	15 ± 10[b]
CD40	80	88	34 ± 8[b]	53 ± 15[b]
CD80	15	13	16 ± 7	13 ± 6
CD86	74	93	9 ± 1	12 ± 2

MA and DC were generated in vitro either from monocytes or hematopoetic progenitors. The phenotype of the various cell populations was determined using flow cytometry. The percentage of cells positive for each marker (as compared to an isotypic control) is indicated. For most markers mean and standard deviation of 4–5 independent experiments are presented, except for CD40, CD80 and CD86 (only measured once on MO-MA and MO-DC). Significant differences, calculated with Student's *t*-test, between expression levels in MO-MA versus MO-DC and in precursor vs. TNF-differentiated HPC-DC respectively are indicated with [a]$P < 10^{-3}$ and [b]$P < 0.05$.

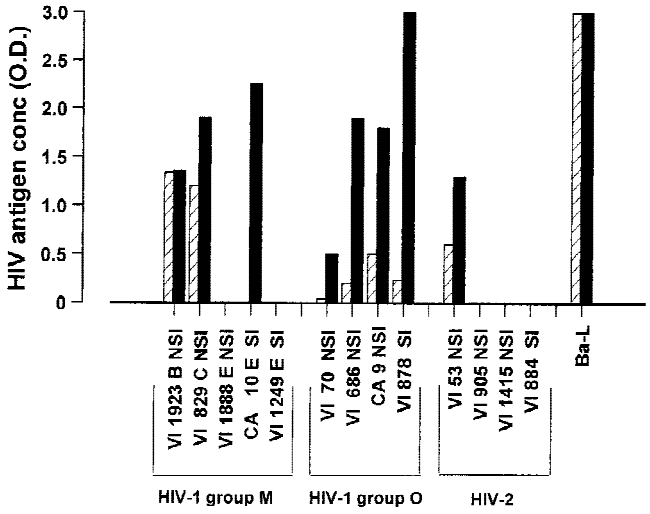

Fig. 1. Infection of precursor HPC- DC with HIV-1 and HIV-2 isolates. Precursor HPC-DC were incubated with 5 HIV-1 group M, 4 group O, 4 HIV-2 isolates and with the reference strain Ba-L at a MOI of 3×10^{-3} for 30 minutes at 4°C. After washing, the cells were cultured in four duplicates in GM-CSF medium. After 9 days, PHA-T blasts in IL-2 medium were added to half of the wells (solid bars) and the other wells were further fed with GM-CSF medium (hatched bars). HIV antigen concentration in the supernatant was measured by ELISA. The mean O.D. of 2–3 independent experiments is shown.

blood HPC. The resulting cell populations were homogeneous for CD13 expression. The proportion of CD4(+) cells was around 40% and CD80 as well as CD86 were present on 10–15% of the cells. CD34 expression was completely lost during the differentiation of HPC to DC and the T cell markers CD3 was never observed. Fifty percent of the DC, generated with TNF, in addition to GM-CSF + IL-4, expressed CD1a and CD40, whereas these markers were only present in around one third of HPC-DC, generated in GM-CSF + IL-4 medium ($P < 0.05$). CD14 expression was present on ±15% of TNF-pretreated HPC-DC, but on ±25% of those generated with GM-CSF + IL-4 only ($P < 0.05$). Thus, the TNF-treated DC had a more differentiated phenotype and will be referred to as "TNF-differentiated HPC-DC." The cells generated without TNF, are less differentiated into DC (less CD1a+ cells), they contain more CD14(+) (= MO-like) cells and will be called "precursor HPC-DC."

To compare the allo-stimulatory capacity of these 4 cell types, a dilution series of the MA or DC was cultured with heterologous CD4 T cells and proliferation was measured after 5 days. Whereas the MA induced only limited T cell proliferation at a high ratio of MA/T cells (>1/10), the allo-stimulatory activity of the 3 different DC preparations became evident at DC/T ratios below 1/100 and was quantitatively similar, despite their phenotypic differences (data not shown).

Progenitor Cell-Derived DC Are More Susceptible to HIV-1 Than to HIV-2 Isolates

Productive infection of precursor HPC-DC was studied with 5 different HIV-1 group M isolates, 4 HIV-1 group O isolates, 4 HIV-2 isolates and Ba-L as a positive control. The virus strains had been previously titrated on PHA T blasts. For the infection of DC a virus dilution, corresponding to a multiplicity of infection (MOI) of 3×10^{-3} was used. The conditions of infection were designed to allow only high affinity interaction between the virus and the cells, by limiting the incubation time to 30 minutes and the temperature was kept at 4°C. On Day 9, non-infected PHA T blasts were added to 2 duplicate wells (to rescue possibly latently integrated virus) and the 2 other duplicates were left with DC only. Each strain was tested in 2 or 3 separate experiments, performed on different days with a different lot of HPC-DC. Data in Figure 1 represent the mean HIV concentration after 3 weeks of culture.

Two out of three NSI HIV-1 M strains (one subtype B and one subtype C) showed productive infection in cultures with precursor HPC-DC and addition of PHA T blasts had only a limited enhancing effect. All 4 HIV-1 group O isolates, including 1 SI and 3 NSI strains, induced low levels of virus production in HPC-DC cultures, that was clearly enhanced by adding PHA T blasts. Of the 4 HIV-2 isolates tested, only one NSI strain (VI 53) could induce measurable HIV production, that was slightly enhanced by T blasts.

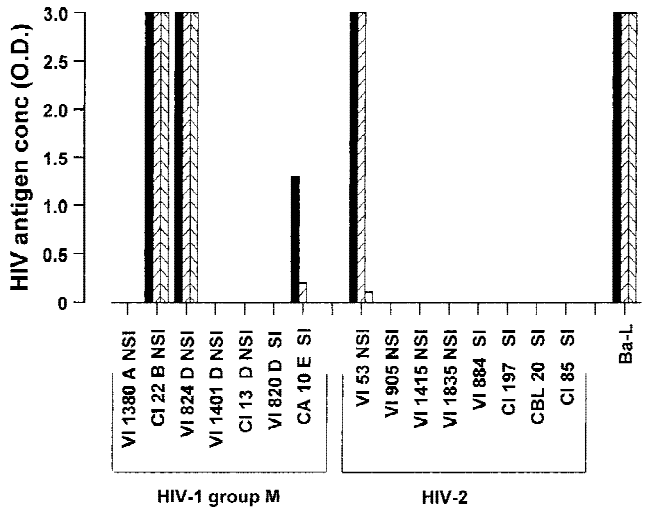

Fig. 2. Infection of Langerhans-like HPC-DC with HIV-1 and HIV-2 isolates. TNF-differentiated-DC were incubated with 7 HIV-1 group M, 8 HIV-2 isolates and with Ba-L at 10^{-2}(solid bars), 10^{-3} (// hatched) and 10^{-4} MOI (\\ hatched) for 2 hours at 37°C. The cells were cultured in duplicate wells in GM-CSF medium for 19 days. The HIV antigen concentration shown is the mean of 2 independent experiments.

In a second series of experiments, the TNF-differentiated HPC-DC were used as target cells. More permissive and physiological conditions of infection, (2 hours at 37°C) and a range of MOI (10^{-2} to 10^{-4}) were used. In this setting productive infection was observed with 2/5 NSI and 1/2 SI HIV-1 group M isolates but with only 1/8 HIV-2 isolates (Fig. 2).

Five isolates (4 HIV-2 and 1 HIV-1) were tested both in precursor HPC-DC (Fig. 1) and TNF-differentiated HPC-DC (Fig. 2). Of the 4 HIV-2 isolates, only VI 53 induced viral production in DC in both cell types. The SI HIV-1 strain CA10 induced a productive infection in TNF-differentiated HPC-DC at 10^{-2} MOI (Fig. 2), whereas addition of PHA blasts was necessary to obtain measurable HIV replication in precursor HPC-DC, using less permissive conditions of infection.

The sensitivity of HPC-DC, prepared under two different sets of conditions, seems to be lower for HIV-2 strains (1/8) as compared to HIV-1 strains (9/15). HIV-1 group O isolates more frequently induced a productive infection than the group M isolates (4/4 vs. 5/11). With regard to phenotypic differences in group M, 4/8 of the NSI and 1/3 of the SI strains induced viral production in HPC-DC. Because this particular SI strain (CA10) was dependent on the CXCR-4 co-receptor and showed some growth in both types of HPC-DC, our data suggest that both precursor and TNF-differentiated HPC-DC functionally express both major co-receptors.

Both Major HIV Co-Receptors Can be Used by the TNF-Differentiated HPC-Dendritic Cells

TNF-differentiated HPC-DC were infected with the reference strains Ba-L and HTLV-III_B in the presence or absence of 10 μg/ml blocking anti-CCR-5 (clone 2D7) or anti-CXCR-4 (clone 12G5). The same antibodies were added only once again, implying that, from Day 7 on, the effective concentration was reduced by 50% each time the medium was replenished. As shown in Figure 3, anti-CCR-5 prevented measurable production of Ba-L during the first week of culture. Thereafter, the virus was present in the supernatant, but at lower levels than in the control cultures. HTLV-III_B production was clearly reduced by anti-CXCR-4, but not at all by anti-CCR5.

This experiment confirms that both co-receptors are functionally present on the HPC-DC; however, high concentrations of blocking co-receptor antibodies both at the time of infection and throughout the first week of culture were ultimately not sufficient to prevent virus production. Attempts to measure the level of expression of the co-receptors, using the PE-labeled 2D7 and 12G5 failed in each type of DC (also the MO-derived cells), most probably because of the high autofluorescence of these long-term cultured cells.

The Growth Characteristics of NSI and SI HIV-1 Clones in Various Models of DC Point to a Differential Susceptibility of MO-Derived as Compared to HPC-Derived DC

Using the same "permissive" inoculation conditions as in Figure 2 (2 hours at 37°C and 10^{-2} to 10^{-4}MOI), the intrinsic sensitivity of MO-MA, MO-DC, precursor HPC-DC and TNF-differentiated DC to productive infection with the four pairs of HIV-1 clones was compared (Table III).

Important differences were evident between the MO-derived and the HPC-derived cells in their relative susceptibility to NSI vs. SI clones. All NSI clones tested induced viral production in MO-DC and MO-MA, whereas these cells seemed almost completely resistant to the SI clones. In contrast, SI clones were able to grow in HPC-DC, but the NSI clones 943-4, 761-1 and 820-1 still induced a higher virus production than their SI counterparts. In contrast, clone 886-6 (SI) was more productive than the NSI 886-1 in both types of HPC-DC.

In general, the precursor HPC-DC and the more differentiated TNF-differentiated HPC-DC were susceptible to a similar spectrum of HIV clones (Table III) and isolates (Fig. 1–2 and other data not shown), but the less differentiated precursor HPC-DC, that also contained more MO-like cells, tended to produce more virus in the supernatant.

HIV Growth in Co-Cultures of Infected TNF-Differentiated HPC-DC and Resting T Cells

To simulate in vivo conditions, where resting CD4 T cells interact with DC soon after infection, purified, non-activated CD4 T cells were added immediately after the experimental infection of the DC. As controls, cultures of infected DC alone and co-cultures of DC with the most sensitive targets, PHA-activated T blast, were set up. To quantify better the effect of T cell addition, the infected DC were dispensed in a dilution

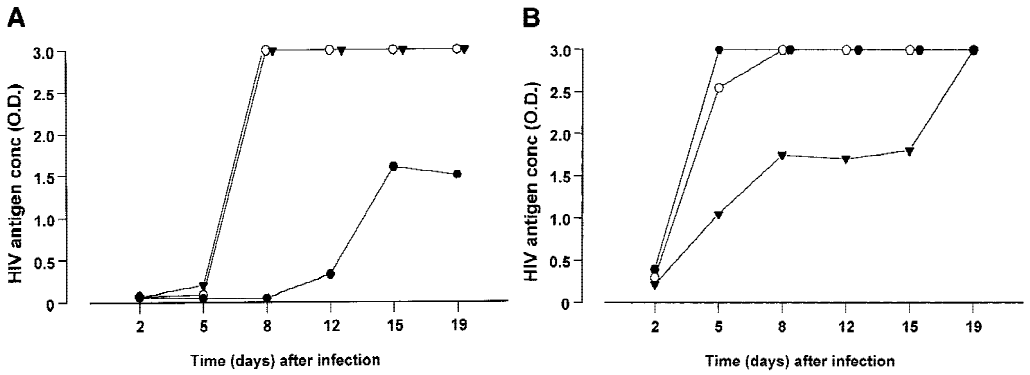

Fig. 3. Effect of anti-chemokine-receptor antibodies on infection of TNF-differentiated HPC-DC. TNF-differentiated HPC-DC were pre-incubated with either medium (open circles), the CCR-5-blocking 2D7 mAb (solid diamonds) or the CXCR-4-blocking 12G5 mAb (inverted solid triangles), both at 10 μg/ml. The reference strains Ba-L at 3×10^{-4} MOI (**A**) and HTLV-III_B at 3×10^{-3} MOI (**B**) were added for 2 hours at 37°C. After washing, the cells were supplemented with GM-CSF medium and the corresponding antibodies, that were added again during the first replenishment (at Day 3). The HIV antigen concentration in the supernatant during the entire culture period is shown.

series ranging from 330 to 0.15 $\times 10^3$/ml, whereas the T cells were always used at 10^6/ml.

In the first pair of experiments the prototypic strains Ba-L and HTLV-III_B were used at 10^{-3} MOI. As expected, Ba-L induced a higher level of productive infection in DC-only cultures. Addition of activated T cells enhanced the production of both Ba-L and HTLV-III_B to a comparable extent. Addition of resting T cells, however, induced viral production with lower numbers of Ba-L infected DC as compared to HLTV-III_B infected DC (Figs. 4A and B). As expected from Table III, the HIV-1 NSI clone 761-1 at 10^{-3} MOI readily-induced virus production in DC alone, whereas the SI clone 761-4 failed to do so; however, if either resting or activated T cells were added, high levels of HIV antigen were found in co-cultures with DC, infected with either clone, even if less than 5000 DC were present (Fig. 4C and D).

In summary, irrespective of the intrinsic "dendrotropism" of the 4 strains/clones tested, high virus production could be induced by addition of PHA T blasts. In three-fourths of these cases (Ba-L, 761-1 and 761-4), resting T cells were similarly efficient as PHA T blasts to increase viral production.

DISCUSSION

To the best of our knowledge, the present study is the first to compare the susceptibility of dendritic cells to primary isolates of HIV-2, HIV-1 group O and HIV-1 group M. To standardize the system, the multiplicity of infection was based on TCID50 values, determined previously in the PHA T blasts, implying that all the results on susceptibility of DC refer to the T blasts as a reference. The hematopoietic progenitor cell-derived DC were less susceptible to HIV-2 isolates than to HIV-1: only 1 of 8 HIV-2 (12.5%) vs. 9 of 15 HIV-1 strains (60%) induced a productive infection. The few available isolates of group O induced 100% infectivity (4/4) as compared to 45% by group M (5/11). The lower susceptibility of HPC-DC to HIV-2 isolates can provide an explanation for the observed preferential spread of HIV-1 in areas of the world were both HIV types are transmitted concomitantly sexually [Kanki et al., 1997; Tarantola and Schwartländer, 1997]. Whether the higher rate of DC infection by the HIV-1 group O, as compared to group M, relates to risk of sexual transmission is presently unclear. Within the panel of HIV-1 group M strains, 1/3 (33%) of the subtype E isolates and 4/9 (44%) isolates of other subtypes productively infected the HPC-DC. This apparent lack of subtype-specificity is in agreement with data obtained in primary DC [Pope et al 1997, Dittmar et al 1997]. The hypothesis of a preferential susceptibility of DC to subtype E [Soto-Ramirez et al., 1996] is therefore not confirmed.

The HPC-DC showed no clear-cut preference for primary isolates of the NSI vs. the SI phenotype: 4/8 of the HIV-1 NSI and 1/3 of the SI viruses productively infected either precursor or TNF-differentiated HPC-DC. Results obtained with primary strains can be misleading, however, because each isolate is a quasispecies with many clones, that may differ in SI capacity and "dendrotropism." Instead of expanding the comparison with isolates, four pairs of biological HIV clones, with opposite (SI-NSI) phenotype, were used to infect HPC-DC. In 3 out of 4 pairs, the NSI/R5 virus more readily infected the HPC-DC. The "atypical" result in the VI-886 clones, where the SI clone (VI 886-6) induced higher viral production in the HPC-DC, may relate to the exceptional use of the CCR-5 co-receptor by this SI clone. Nevertheless, the fact that HPC-DC showed some susceptibility to both SI/X4 and NSI/R5 clones suggested that both co-receptors are functionally present. This hypothesis was supported by the demonstration that the prototypic NSI/R5 strain Ba-L and the prototypic SI/X4 strain HTLV-III_B were both able to productively infect HPC-DC. Moreover, these infections were co-receptor-mediated, because receptor-

TABLE III. Intrinsic Susceptibility of MO-Derived MA and DC and of HPC-Derived DC to Various HIV-1 Clones

Cells of origin differentiated cell type	Peripheral blood monocytes		Cord blood HPC	
	MO-MA	MO-DC	Precursor HPC-DC	Differentiated HPC-DC
NSI clones				
VI 820-1				
10^{-2} MOI	ND	2.9	2.9	1.6
10^{-3}		1.5	1.0	0.5
10^{-4}		0	0	0
VI 886-1				
10^{-2} MOI	0	1.5	1.2	0
10^{-3}	0	0	0	0
10^{-4}	0	0	0	0
VI 761-1				
10^{-2} MOI	2.1	2.3	2.9	2.9
10^{-3}	2.0	2.3	2.9	2.9
10^{-4}	0.7	2.0	2.9	2.9
VI-943-4				
10^{-2} MOI	2.0	2.8	1.5	1.5
10^{-3}	0	0	0	0
10^{-4}	0	0	0	0
SI clones				
VI-820-4				
10^{-2} MOI	ND	0.1	1.2	0.4
10^{-3}		0	0	0
10^{-4}		0	0	0
VI 886-6				
10^{-2} MOI	0.1	0	1.1	0.5
10^{-3}	0	0	0.4	0.1
10^{-4}	0	0	0	0
VI 761-4				
10^{-2} MOI	0	0	2.2	0.2
10^{-3}	0	0	0.6	0
10^{-4}	0	0	0	0
VI 943-1				
10^{-2} MOI	0	0	0.6	0
10^{-3}	0	0	0	0
10^{-4}	0	0	0	0

Four pairs of NS/SI HIV-1 clones were incubated with MA and DC for 2 hours at 37°C at the indicated MOIs. The MA and DC were cultured in duplicate 500 μl cultures in GM-CSF-containing medium for 19 days and the final HIV antigen concentration in the supernatant was determined by ELISA. The results shown are the mean O.D. of 2 experiments. ND = not determined.

blocking anti-CCR-5 and anti-CXCR-4 reduced the virus production of Ba-L and HTLV-III_B respectively. The preference for NSI/R5 clones in comparison to SI/X4 viruses was more clear-cut with MO-MA and MO-DC than with HPC-DC.

Some researchers have shown that dendritic cells of various origins are able to replicate both NSI/R5 and SI/X4 [Blauvelt et al., 1997 in MO-DC; Carr et al., 1998 in HPC-DC; Langhoff et al., 1991 in primary blood DC). Others have claimed, however, that DC were essentially not susceptible to SI strains, [Chehimi et al., 1993 in primary blood DC; Warren et al., 1997 in differentiated HPC-DC]. These seemingly conflicting observations may be explained by the origin of the DC, their maturation stage or the cytokine environment, that influence co-receptor expression and function [Cameron et al., 1994; Ludewig et al.,1995; Blauvelt et al., 1997; Triozzi and Aldrich, 1997; Warren et al., 1997; Carr et al., 1998; Granelli-Piperno et al., 1998]. In addition, contamination with macrophages or T cells can also contribute to variable results. Whereas T cell contamination was carefully excluded in the present study, monocyte-like (CD14+) cells are certainly present in the HPC-DC preparations. Clearly, it is not evident from the presented data in which cell subset of HPC-derived cells the productive HIV infection takes place. During the revision of this manuscript a study appeared that addresses this question [Wang et al., 1999]. These authors used both Ba-L and HTLV-IIIB to infect HPC-DC and showed that, at the peak of infection, both the CD1a(+) and the CD14(+) subsets express HIV in their cytoplasm.

The intrinsic susceptibility of DC for HIV probably constitutes a first limiting step to sexual transmission, but the efficiency of the transfer to T cells is a second critical event in the dissemination of the virus. Several researchers have suggested that the DC-to-T cell transfer and the productive infection of the latter cells does not necessitate a definite replication in DC, but HIV binding to the surface of the DC probably suffices for the transfer [Tsunetsugu-Yokota et al., 1995; Weissman et al., 1995; Weissman et al., 1996; Granelli-

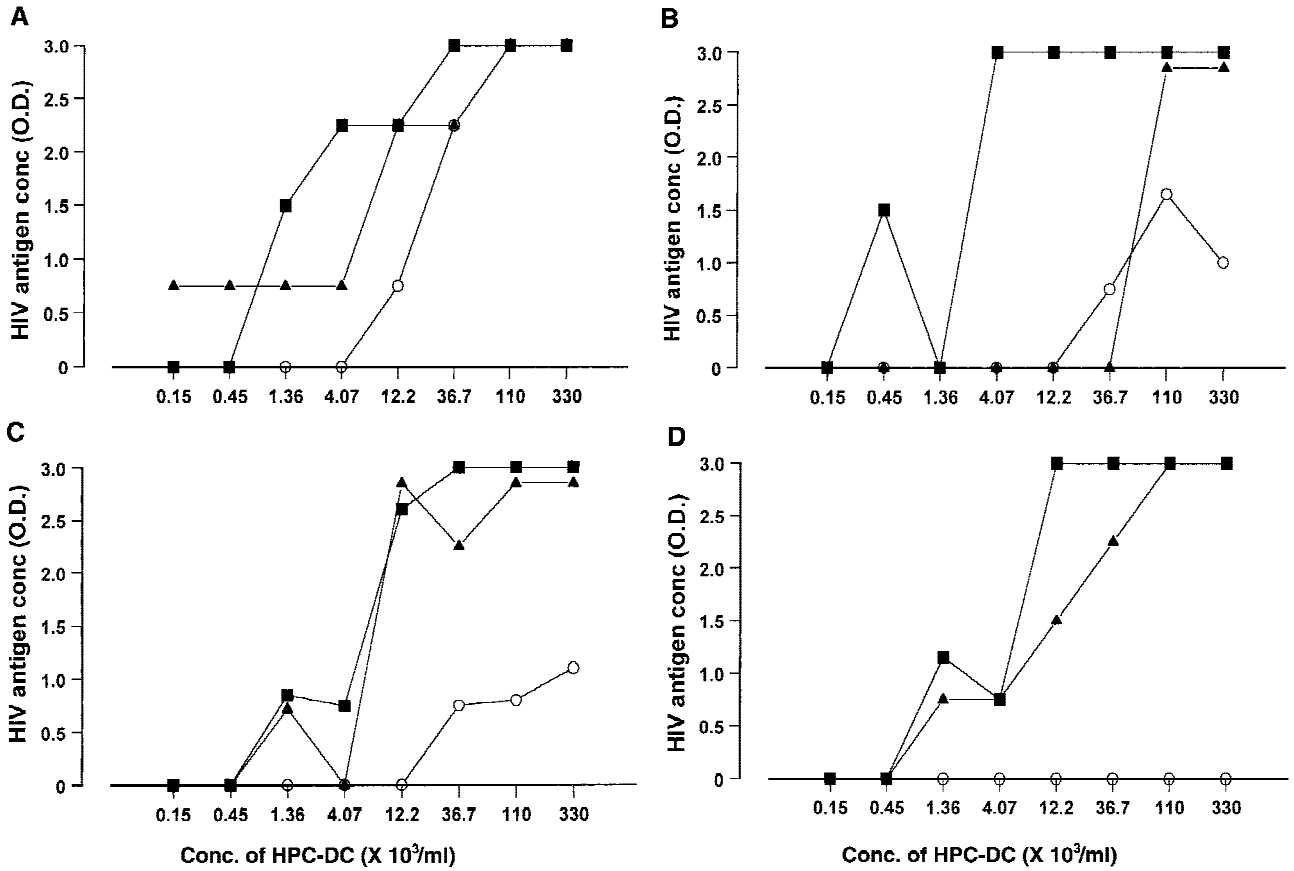

Fig. 4. Enhancing effect of T cells on virus production after infection of TNF-differentiated HPC-DC. TNF-differentiated HPC-DC were infected with 10^{-3} MOI Ba-L (**A**), $HTLV\text{-}III_B$ (**B**), clone 761.1 (**C**) and clone 761.4 (**D**) for 2 hours at 37°C. After 5 washes, the TNF-differentiated HPC-DC were dispensed in a ⅓ dilution series (final concentrations represented on the x-axis) and either cultured alone (open circles), with 10^6 activated PHA T blasts per ml (solid squares) or with 10^6 resting CD4 T cells per ml (solid triangles). The virus production after 19 days of culture was measured. The result shown is the mean of 4 observations (2 duplicates ×2 independent experiments).

Piperno et al., 1998]. To investigate further this point in our model, HIV variants, showing different levels of productive infection in DC themselves, were compared for their HIV antigen production in co-cultures with uninfected T cells. Both PHA-preactivated and originally resting T cells clearly enhanced viral production. In our limited series neither the NSI/SI phenotype, nor the co-receptor usage (R5/X4), nor the overall "intrinsic dendrotropism" could predict the enhancing effect of T cells on virus production. Conceivably, the increased virus output in DC-T co-cultures could be the result of a bi-directional interaction. "Resting" T cells are activated to some extent by DC and thus become more susceptible to infection. Alternatively, during their interaction with DC, the T cells may also stimulate the DC (e.g., via CD40-ligand) and thus favor virus replication in the DC themselves [Ludewig et al., 1996]. In a recent experiment we used fluorescence techniques to distinguish between these two possibilities and obtained conclusive evidence of massive transfer to T cells, without increase of viral expression in DC themselves (manuscript in preparation)

Clearly, MO-derived as well as HPC-derived DC are relatively easy to prepare in large quantities, they have common characteristics (e.g., similar allo-stimulatory capacity) and both models provide useful tools for research on DC-HIV interactions. Nevertheless, these two models are distinct in various aspects. The susceptibility of MO-DC and HPC-DC to various HIV clones differs, with MO-derived cells showing a more clear-cut NSI over SI preference. The phenotype of the MO-DC is more homogeneous and typical of peripheral (dermal) immature DC, whereas the phenotypic characteristics of HPC-derived cells suggest that both DC and MO-like cells are present. Overall, the MO-derived cells seem the better alternative for research on a larger scale. Practical advantages of the MO-DC include the general availability of buffy coats and the shorter differentiation time. Moreover, as compared to HPC-DC, the MO-DC are more analogous to the presumed first target during sexual transmission.

In summary, HIV-2 isolates were less infectious in DC than HIV-1 isolates and HIV-1 group O strains were more "dendrotropic" than HIV-1 group M isolates. The subtype of the group M isolates had no effect on their infectivity in HPC-DC. Especially at the level of biological clones, NSI/R5 variants were more infectious in DC than the SI/X4 counterparts. The latter distinc-

tion was much more evident, however, in MO-DC as compared to HPC-DC. Finally, the lower intrinsic "dendrotropism" of some HIV variants in HPC-DC could be "corrected" to high levels of productive infection in cocultures with resting T cells. Overall, our data indicate that dendrotropism is related to HIV genotype and phenotype, but other, as yet unknown, factors must also have a role. Model systems like those described above are suitable to analyze the complex interaction between HIV, DC and T cells, that is important for the understanding of early events during sexual transmission.

ACKNOWLEDGMENTS

We thank Dr. Mertens from the Antwerp Red Cross Blood Transfusion Centre for giving us access to buffy coats from healthy blood donors. These studies were supported by grant 3.0226.96 from FWO Vlaanderen (Fund for Scientific Research Flanders).

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